REVIEW

Biocompatibility of dental materials used in contemporary endodontic therapy: a review. Part 2. Root-canal-filling materials

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Abstract

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Root-canal-filling materials are either placed directly onto vital periapical tissues or may leach through dentine. The tissue response to these materials therefore becomes important and may influence the outcome of endodontic treatment. This paper is a review of the biocompatibility of contemporary orthograde and retrograde root-canal-filling materials.

Keywords: biocompatibility, biomaterials, endodontics.

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Orthograde root-canal-filling materials

Solid filling materials

Gutta-percha

Gutta-percha is the main filling material used in root-canal treatment although it only forms about 20% of the composition of modern gutta-percha cones. Zinc oxide is the main component (60–70%) of the cone and provides a major part of the radiopacity. The remaining 10% is not specified, as it is proprietary information but consists of a mixture of resins, waxes and metal sulphates (Spångberg 1999).

Gutta-percha is considered to have acceptable biocompatibility with a low degree of toxicity, a view based on findings in animal studies involving implanted pieces of gutta-percha (Spångberg 1969a, Wolfson & Seltzer 1975). These studies describe the formation of a collagenous capsule around the implants with very little or no inflammatory host response. Gutta-percha cones have however, been shown to be cytotoxic in *in vitro* tests. Spångberg (1969a) suggested that toxic agents bound to water-insoluble substances are responsible for the toxicity of gutta-percha cones. These substances are set free when gutta-percha is dispersed into small particulate fractions.

Wolfson & Seltzer (1975) found that with the exception of a calcium hydroxide and a chloroform-containing product, the toxic effects of natural occurring gutta-percha (*trans*-polyisoprene) are similar to those of commercial gutta-percha. Munaco *et al.* (1978) and Pascon & Spångberg (1990) regarded the cytotoxic effect of commercial gutta-percha to be due to the high content of zinc oxide. Contrary to this belief, results from the study by Sunzel *et al.* (1997) showed that the addition of zinc reduced the toxicity of rosin and resin acid and appeared to be cytoprotective.

Complement activation was applied as a parameter to determine the inflammatory potential of four different brands of gutta-percha and nine ingredients of one of those products (ZnO, TiO_2, $BaSO_4$) *in vitro* (Serene *et al.* 1988). Each investigated brand of gutta-percha and all individual gutta-percha components stimulated the complement system. It was concluded from this data that overextension of gutta-percha might contribute to pain and periapical inflammation although this has never been shown clinically.

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Holland *et al.* (1982) investigated the long-term reaction of rat connective tissue to silver and gutta-percha points over a period of 1 year. One brand of gutta-percha and the silver points were well tolerated. The other brand of gutta-percha points caused pronounced effects with thick fibrous capsules and severe chronic inflammation of the surrounding connective tissue. This observation is in line with the inflammatory potential of gutta-percha as shown in the study by Serene *et al.* (1988).

In a study by Sjögren *et al.* (1995) the tissue reaction to gutta-percha in the form of large, fine and rosin-chloroform-dissolved particles was tested. Large gutta-percha particles were well encapsulated and the surrounding tissue was free of inflammation. Fine particles and those treated by rosin-chloroform evoked an intense, localized tissue response, characterized by the presence of macrophages and multinucleated giant cells. The authors concluded that the size and surface character of gutta-percha determine the tissue reaction to the material.

Calcium hydroxide-containing gutta-percha points and their efficacy comparable with calcium hydroxide pastes have been demonstrated (Holland *et al.* 1996). In an *in vitro* study by Podbielski *et al.* (2000) calcium hydroxide containing gutta-percha points demonstrated good inhibitory action on the bacterial growth of three of the four test organisms. Iodoform gutta-percha cones, introduced by Martin & Martin (1999) had a negligible effect on *Enterococcus faecalis*, but demonstrated a significant inhibitory effect on *Streptococcus sanguis* (Silver *et al.* 2000).

Warm gutta-percha techniques impose the additional complication of heat generated during obturation which may have a deleterious effect on the periodontium. A temperature rise of 10 °C above normal body temperature is regarded as a critical level at which irreversible damage to periodontal tissues can occur (Gutmann *et al.* 1987, Saunders 1990). Studies have shown that the temperature hardly ever exceeded an increase of 10 °C at the external root surface during themoplasticized gutta-percha obturation techniques (Gutmann *et al.* 1987, Silver *et al.* 1999, Sweatman *et al.* 2001) and as such should not pose a problem.

Obturation with gutta-percha requires the use of sealing cement and although gutta-percha has been shown to be cytotoxic, the sealers are normally the most toxic element of the filling.

Sealers and cements

Zinc oxide–eugenol sealers

These sealers are simply zinc oxide–eugenol (ZnOE) cements modified for endodontic use. The liquid for these materials is eugenol whilst the powder contains finely sifted ZnO to enhance the flow of the cement. In a study by Serene *et al.* (1988), it was found that ZnOE sealers activated the complement system and thus an inflammatory reaction. Additionally, Guigand *et al.* (1999) found these sealers to be severely cytotoxic in fibroblast cultures. These properties are mainly attributed to the eugenol component. However, ZnOE sealers easily lend themselves to the addition of chemicals, which may contribute to its cytotoxicity. Rosin or Canada balsam is added for greater dentine adhesion, *para*formaldehyde for antimicrobial and mummifying effects, germicides for antiseptic action and corticosteroids for suppression of inflammation.

Rosins are derived from a variety of conifers and are composed of approximately 90% resin acids. The remaining parts are volatile and non-volatile compounds such as terpene alcohol, aldehydes and hydrocarbons. The carbon group of resin acids is lipophilic, affecting the lipids in cell membranes and increasing the cell membrane permeability. The resin acids are both antimicrobial and cytotoxic (Söderberg 1990).

Zinc oxide is effective as an antimicrobial agent (Sunzel *et al.* 1990) and has been shown to provide cytoprotection to tissue cells. Interactive toxic effects between zinc and rosin or resin acid was investigated using human polymorphonuclear leucocytes and gingival fibroblasts. Rosin and resin acids were highly cytotoxic depending on the concentration. The addition of zinc clearly reduced rosin and resin acid toxicity proportionally with increasing zinc concentration (Sunzel *et al.* 1997).

Non-specific histocompatibility tests showed that eugenol-based sealers elicited a pronounced tissue irritation (Kolokuris *et al.* 1998a, Gulati *et al.* 1991). Two ZnOE sealers (Sealite; Pierre Rolland Co., Merignac, France), Kerr Pulp Canal Sealer (Kerr, Romulus, MI, USA) were, however, rated biocompatible, based on histologic response, when implanted into the mandibular bone of rabbits (Pertot *et al.* 1992). Kolokuris *et al.* (1998a) found that subcutaneous injection of a ZnOE sealer (Roth 811 (Roth Drug Co., Chicago, IL)) into rats affected the normal concentrations of Zn, Ca & Cu in various organs, e.g. heart, brain, liver and kidney. These results suggest that ZnOE sealers may release considerable amounts of these compounds, which are then deposited in vital organs.

A comparative study on the effects of one calcium phosphate cement (CPC) and two ZnOE sealers was performed with intentional overfilling in monkeys over a 6-month period. The CPC caused only mild inflammatory periapical reactions in the initial study period whereas both ZnOE sealers (Grossman's sealer; Pharmacy, Glasgow, Royal Infirmary, UK) and N2 (Indrag-Agsa, Losone, Switzerland) were severely irritating over the full 6-month duration (Hong *et al.* 1991). Despite the antimicrobial effect of rosin and zinc oxide on facultative bacteria, zinc-releasing materials such as gutta-percha and ZnOE sealers, especially those releasing *para*formaldehyde, have been associated with maxillary sinus aspergillosis (Beck-Mannagetta & Necek 1986, Kobayashi 1995, Odell & Pertl 1995). It was hypothesized that *para*formaldehyde penetrates the sinus through overfilled sealer and may cause tissue irritation and local necrosis of the sinus mucosa. Subsequently, zinc oxide is released from the sealer, which may then promote growth of inhaled *Aspergillus* conidia (Beck-Mannagetta & Necek 1986).

The setting of zinc oxide–eugenol cements [such as Rickert's sealer (Kerr, Romulus, MI, USA), U/P-Grossman's sealer (Pharmacy, Glasgow, Royal Infirmary, UK), Tubli-seal (Kerr, Romulus, MI, USA)] is a chemical process combined with physical embedding of zinc oxide in a matrix of zinc eugenolate. Analyses of the release of eugenol from set ZnOE cement showed that the oil is available only as the result of surface hydrolysis of the chelate (Wilson & Batchelor 1970, Becker *et al.* 1983, Hume 1984). Samples of ZnOE placed into saline showed an immediate release of eugenol from the ZnOE surface with the highest rate of release in the first seconds after contact, the release rate declined exponentially thereafter. In contrast, the release rate through intervening dentine was found to be entirely different. The release of eugenol was found to be much slower and could only be detected after several hours, it peaked after about a day and then declined slowly over several weeks. A sustained release occurred with the establishment of a relatively stable concentration gradient across the dentine which persisted for several months (Hume 1988). On the basis of data on this concentration gradient, Hume (1988) predicted the various pharmacological and toxicological actions of eugenol. In the dentine immediately beneath the ZnOE, the concentration of eugenol is sufficient to inhibit bacterial metabolism whilst the concentration in more remote dentine would be below the threshold for killing mammalian cells but at a sufficient level to exhibit pharmacological properties for which eugenol is renowned such as inhibition of nerve action potential (Brodin & Ørstavik 1983) and prostaglandin synthesis (Dewhirst 1980). If ZnOE contacts wetter tissue the release is more rapid, leading to the development of concentrations sufficient to kill cells. This explains the toxic effects of ZnOE when applied to wet tissues or to cells in culture.

The toxic potency of eugenol has been demonstrated by Araki *et al.* (1993a, 1994) who found that the sealer, Canals (Syowa Yakuhin Kako Ltd., Tokyo, Japan), with eugenol as the liquid component was significantly more cytotoxic in permanent L929 cells and primary human periodontal ligament fibroblasts than the material, Canals-N (Syowa Yakuhin Kako Ltd., Tokyo, Japan), with an identical powder as Canals but with fatty acids replacing eugenol as the liquid component. Similarly, it has been shown that the eugenol component of a sealer was highly cytotoxic to primary human periodontal ligament fibroblasts whereas the other sealer ingredients TiO_2, ZnO and $BaSO_4$ caused only slight toxic effects (Klaiber *et al.* 1981). The effect of eugenol on the adherence of immunocompetent cells to substrate was studied with peritoneal macrophages from Wistar rats. Even high dilutions (1 : 1000) of this phenol derivative significantly reduced the adhesion of macrophages. Thus, eugenol could inhibit macrophage function and may influence inflammatory reactions in the periapical tissues (Segura & Jmenez-Rubio 1998). The pronounced antibacterial effect of the ZnOE sealer is very likely due to eugenol (Al-Khatib *et al.* 1990, Mickel & Wright 1999). For a long time, it has been common to mix formaldehyde into some endodontic sealers (Endomethasone (Septodont, Saint-Maur, France) and N_2 (Indrag-Agsa, Losone, Switzerland)). Formaldehyde adds to the already toxic effect of eugenol and prevents or inhibits healing.

Calcium hydroxide-containing sealers

Several sealers, e.g. Sealapex (Kerr, Romulus, MI, USA), CRCS (Hygenic, Akron, USA) and Apexit (Vivadent Schaan, Liechtenstein) have been marketed which claim the benefits of the biological effects of added $Ca(OH)_2$. In order to be therapeutically effective, calcium hydroxide must be dissociated into Ca^{2+} and OH^- ions. Therefore, to be therapeutic an endodontic sealer based on calcium hydroxide must release these ions which may affect the structural integrity of the sealer and compromise the long-term seal.

In a study of diffusion of hydroxyl ions into surrounding dentine after root filling with Sealapex and Apexit, no traces were found in teeth filled with Apexit. Some hydroxyl ions could be detected in the dentine close to the root filling with Sealapex (Staehle *et al.* 1995). In a similar study of calcium and hydroxyl ion release from Sealapex and CRCS, negligible release was noted from CRCS, whilst Sealapex released more ions but disintegrated in the process (Tagger *et al.* 1988). *In vivo* studies have demonstrated that Sealapex and CRCS easily disintegrate in the tissue (Soares *et al.* 1990), and both may cause chronic inflammation (Tronstad *et al.* 1988).

Calcium hydroxide sealers are generally characterized as having good cytocompatibility (Feiglin 1987, Beltes *et al.* 1995, Economides *et al.* 1995, Vajrabhaya & Sithisan 1997, Vajrabhaya *et al.* 1997, Geurtsen *et al.* 1998, Osorio *et al.* 1998, Telli *et al.* 1999, Ersev *et al.* 1999). However, unsatisfactory results were found with CRCS in several studies (Tronstad *et al.* 1988, Yesilsoy *et al.* 1988, Boiesen & Brodin 1991, Bezerra *et al.* 1997). CRCS is considered a standard ZnOE sealer by various authors though it also contains $Ca(OH)_2$. The $Ca(OH)_2$-containing sealer Apexit showed no mutagenic potential in a study by Schweikl & Schmalz (1991).

Nonspecific histocompatibility tests on calcium hydroxide-based sealers were variable after subcutaneous implantation or intraperitoneal injection in mice. Inflammation and tissue necrosis were especially prominent during the initial period after application.

Neurotoxic effects were investigated with isolated nerves *in vitro.* Rat phrenic nerves were treated for 1.5–30 min with two calcium hydroxide-containing sealers, CRCS and Sealapex. Short-term contact resulted in a reversible inhibition of the nerves whereas neural conduction was irreversibly inhibited after 30 min by both sealers (Boiesen & Brodin 1991).

Specific histocompatibility was tested in dog root canals to compare the periapical reaction to four $Ca(OH)_2$-containing sealers (Leonardo *et al.* 1997). After 6 months, no inflammatory lesions and complete sealing of the apical region of the root canals were found with Sealapex. However, incomplete sealing of the filled canals and periapical inflammation were identified when CRCS, Apexit or Sealer 26 (Dentsply, Petrópolis, RJ, Brazil) were used. The inflammatory reactions were related to an incomplete adaptation of the root-canal fillings (Leonardo *et al.* 1997). These results indicate that an incomplete adaptation may increase *in vivo* degradation of the sealer with subsequent periapical irritation due to elevated quantities of released substances, or it may favour microbial leakage contributing to periapical inflammation.

Finally, calcium hydroxide and Sealapex impaired the status of the periapical tissue when the materials were extruded through the apex. No adverse effects were found with root fillings terminating at the anatomical foramen. These observations suggest that mechanical stimuli of the periapical tissue may influence the compatibility of a root-canal filling *in vivo* (Sonat *et al.* 1990).

Formaldehyde-containing sealers

A large group of sealer/cements, including the commonly used Endomethasone (Deproco UK Ltd., Surrey, UK), Riebler's paste (Amubarut; Wera Karl, Biesingen, Germany) and N2 (Indrag-Agsa, Losone, Switzerland) contain substantial amounts of *para*formaldehyde. Of these, N2 has been most studied. The contents of N2 are not much different from other *para*formaldehyde-containing sealers as far as toxicity is concerned and it is basically a zinc oxide–eugenol sealer, the exact composition of which has changed over the years. Thus, the significant lead oxide content (England *et al.* 1980), as well as the smaller amounts of organic mercury that were formerly major components of N2 are often missing from modern formulations although it still contains large amounts (4–8% v/v) of formaldehyde. It loses substantial volume when exposed to fluid (Grossman 1978) with this volume loss being exacerbated when it also contains hydrocortisone (e.g. Endomethasone; Septodont, St Maur, France). N2 seals well when used with a core (Brown *et al.* 1979). It has been found to be very toxic, both *in vitro* (Spångberg & Langeland 1973) and *in vivo* (Spångberg 1969a, 1974). Coagulation necrosis is normally observed within a very short time period and reaches a maximum in less than 3 days. Since the tissue is impregnated with formaldehyde, repair of the necrosis is a slow process, often taking months to occur. In time, the formaldehyde is flushed from the necrotic tissue (Block *et al.* 1980, Araki *et al.* 1993b), followed by either bacterial invasion or, if the blood supply to the tissue is still adequate, repair (Spångberg 1969a, 1974). In clinical setting, this untoward tissue reaction can be seen as localized inflammatory reactions in the periapical tissue (Engström & Spångberg 1969b).

Cases of hypersensitivity reactions, presumably as a result of systemic exposure to formaldehyde, have been reported following root-canal treatment (Forman & Ord 1986, Fehr *et al.* 1992). In addition to its cytotoxic properties, formaldehyde is known to be both mutagenic (Goldmacher & Thilly 1983) as well as carcinogenic (Swenberg *et al.* 1980), though these effects have not been attributed to formaldehyde release from endodontic materials (Lewis & Chestner 1981). Some epoxy resin sealers have been reported to release formaldehyde when freshly prepared and during setting (Koch 1999) and these will be discussed under resin sealers.

Chloroform-based sealers

Chloroform-based sealers such as rosin-chloroform (Callahan 1914), Chloropercha (Tanrac Ltd., Gävle, Sweden), Kloropercha (Svenska AB, Stockholm, Sweden) and Kloroperka N-Ø (Union Broach Corp., Long Island City, NY, USA) are common. Chloropercha consists of white gutta-percha and chloroform and derives its toxicity from the chloroform component. Rosin chloroform contains 5–8% of various rosins that are toxic. Thus, after the evaporation/absorption of chloroform, the resin continues to be irritating (Spångberg & Langeland 1973, Sjögren *et al*. 1995). Kloroperka N-Ø powder contains approximately 20% white gutta-percha and 50% zinc oxide. The remaining components are Canada balsam and rosins which may act as irritants after the loss of the chloroform. However, the combination with zinc oxide should provide a significant level of cytoprotection in clinical use (Söderberg 1990).

The general use of chloroform has been substantially curtailed in recent years due to concerns about its toxicity, however, the amount normally used in endodontics is insignificant and poses no health hazard. Nevertheless, it is important to reduce the vaporization during use because chloroform is highly volatile. When used for softening of gutta-percha during the removal of old root-canal fillings or for the chloroform dip root-canal-filling technique the chloroform should be dispensed through a syringe and hypodermic needle (Donelly 1993). For other uses the exposure time, amount used and chloroform surface exposed should be minimized.

Halothane and turpentine may be used as chloroform substitutes. Halothane is less effective than chloroform in softening gutta-percha, is hepatotoxic like chloroform and has a higher local toxicity than chloroform. Turpentine is not carcinogenic but may cause allergies, it has high local toxicity and dissolves gutta-percha poorly. Several other chloroform substitutes have been described for use in endodontic treatment procedures (Uemura *et al*. 1997, Hansen 1998). However, with careful workplace hygiene there is little risk associated with the use of chloroform in endodontics (Barbosa *et al*. 1994).

Polymers

Most of the newer sealers on the market are polymers. They include epoxy resin sealers, e.g. AH26 and AH Plus (DeTrey Dentsply, Konstanz, Germany), methacrylate-based sealers, e.g. polyhydroxy-ethylmethacrylate (Hydron, NPD Dental Systems Inc., New Brunswick, NJ, USA), polyvinyl-based sealers (Diaket-A, ESPE-Premier, Norristown, PA, USA) and polydimethylsiloxane (Roeko-Seal, Langenau, Germany). Like most sealers AH26 (DeTrey Dentsply) is very toxic when freshly prepared (Spångberg 1969b, Pascon *et al*. 1991). The toxicity of AH26 sealer is attributed to the release of a very small amount of formaldehyde as a result of the chemical setting process. This amount of brief release of formaldehyde, however, is thousands times lower than the long-term release from conventional formaldehyde-containing sealers such as N2 (Spångberg *et al*. 1993) but significantly higher than the amount released by AH Plus (Spångberg *et al*. 1993, Cohen *et al*. 1998, Leonardo *et al*. 1999a).

AH26 contains a catalyst/disinfective agent, hexamethylenetetramine (methenamine), which is hydrolysed to ammonia and formaldehyde. It is a very hydrophilic material and formaldehyde is processed only by hydrolytic cleavage of hexamethylenetetramine (methenamine). The surface of the freshly mixed material in contact with water apparently determines the amount of formaldehyde released (Koch 1999). Koch (1999) studied the formaldehyde release from three different root-canal sealers (AH26, Amubarut a phenol resin, and N2), and demonstrated that all materials showed the highest release of formaldehyde in the freshly mixed samples. The release of formaldehyde decreased after setting for 48 h. No further decrease was seen after storage for 2 weeks in the case of N2, whilst AH26 released only insignificant amounts after 2 weeks.

After the initial setting, AH26 exerts little toxic effect *in vitro* and *in vivo* (Bergdahl *et al*. 1974, Wennberg *et al*. 1974, Pascon & Spångberg 1990). Azar *et al*. (2000) demonstrated early cytotoxic effects of AH26 on fibroblasts lasting for 1 week followed by a substantial reduction in cytotoxicity. The cytotoxicity of AH Plus was confined to the early period of the experiment and was no longer detectable after 4 h after mixing. AH26 and AH Plus have been rated as highly, moderately or slightly toxic in various studies involving different tests (Klaiber *et al*. 1981, Meryon & Brook 1990, Vajrabhaya *et al*. 1997, Geurtsen *et al*. 1998, Koulaouzidou *et al*. 1998, Telli *et al*. 1999, Cohen *et al*. 2000). AH Plus exhibited a lower cytotoxicity potential compared to AH26 in the study by Huang *et al*. (2002).

Schweikl *et al*. (1995) studied the genotoxicity of AH26 and its components using the V79/*hprt* mammalian cell mutation assay. Unset sealer was clearly genotoxic, however, the mutation frequency decreased proportionally to increased setting time. Both AH26 and AH Plus caused a dose-dependent increase in genotoxicity in the study by Huang *et al*. 2002). AH26 liquid was also distinctly mutagenic. It was concluded that two mutagenic ingredients – bisphenol-A-diglycidyl-ether and formaldehyde – were the causative agents. Silver-free AH26 revealed a weak positive mutagenic response to the Ames test (Ersev *et al*. 1999). Other investigators found AH26 to be mutagenic up to 1 month after setting (Stea *et al.* 1994, Heil *et al.* 1996, Jukic *et al.* 2000). It was speculated that this 'long-term' genotoxic action was due to a derivative of bisphenol-A-diglycidyl-ether. Contradictory data have been reported about the mutagenic potential of AH Plus in the unset and set condition. A comprehensive screening using four *in vitro* and *in vivo* assays (*umu*, Ames, DIT, AFE) yielded no indication that this sealer may cause mutagenicity in the set condition (Leyhausen *et al.* 1999). However, using the Ames test, a weak mutagenic activity was found in the unset condition and up to 1 day after mixing (Schweikl *et al.* 1998, Jukic *et al.* 2000).

In a long-term specific histocompatibility study in baboons by Pascon *et al.* (1991), AH26 caused severe periapical inflammation after 1–7 days. After periods of 2–3 years, however, AH26 was found to cause only slight irritation. An *in vivo* study in dogs' premolars (Leonardo *et al.* 1999b) demonstrated hard tissue formation apically to the AH Plus in 14 of the 16 roots analysed. Inflammatory cells or areas of necrosis were not associated with AH Plus. AH26 was found to be markedly antimicrobial particularly against *Porphyromonas endodontalis*, an effect that was ascribed to the formaldehyde release during the initial period after mixing (Spångberg *et al.* 1993).

Diaket is a polyketone compound containing vinyl polymers mixed with zinc oxide and bismuth phosphate (Schmitt 1951). In the studies by Spångberg (1969a,b,c,d) it was found highly toxic *in vitro*, causing extensive tissue necrosis and long-lasting irritation. Olsson *et al.* (1981a,b) have shown data suggesting mild tissue reactions after longer periods of time and Olsson & Wennberg (1985) suggested marked reduction in tissue irritating effect after 2 weeks. Results from the study by Ørstavik & Mjör (1988) also showed Diaket to have a favourable biocompatibility especially in comparison to the other tested sealers. Diaket showed no mutagenic potential in the study by Schweikl & Schmalz (1991). Biocompatibility has been assessed when the material was embedded in bone. Nencka *et al.* (1995) implanted Diaket into the tibia of rats and observed that it caused a severe inflammatory reaction at 3 days, with a gradual decrease in intensity until no reaction was seen at 180 days. These results suggest that Diaket has acceptable biocompatibility.

Glass ionomer sealers

Glass ionomer cements have been introduced as endodontic sealers (e.g. Ketac-Endo, ESPE GMBH & Co., KG, Seefeld/Oberbay, Germany), as root-end-filling materials (Zetterqvist *et al.* 1991, Jesslén *et al.* 1995) and as a perforation repair material (Alhadainy & Himel 1993).

Ketac-Endo (ESPE GMBH & Co.), a glass ionomer cement modified for endodontic use, is known to cause minor tissue irritation (Zetterqvist *et al.* 1987, Zetterqvist *et al.* 1988) and low toxicity *in vitro* (Pissiotis *et al.* 1991). Kolokuris *et al.* 1996) implanted Ketac-Endo in Teflon tubes into the subcutaneous tissue of Wistar–Furth rats. A mild inflammatory reaction was seen after 5 days of implantation. The intensity of the inflammatory reaction diminished by the 15th day and this reduction continued progressively through the 60th and 120th day, thus showing good biocompatibility and confirming the findings of Zetterqvist *et al.* (1987, 1988). It had little cytotoxic effect on L-929 cells after a setting time of 24 h and 1 week, respectively, and showed no mutagenic potency in the Ames test (Ersev *et al.* 1999). Ketac-Endo showed antibacterial activity to *Enterococcus faecalis* after 24 h with an increased activity after 7 days (Heling & Chandler 1996). In a study by Leonardo *et al.* (2000), Ketac-Endo was antibacterial to all seven tested bacterial strains.

Root-end-filling materials

Presently root-end fillings are explicitly excluded from the scope of the ISO technical standards for root-canal-filling materials and they have not yet been subjected to standardization (unpublished data).

Gutta-percha

The use of gutta-percha to fill root-end preparations has been described but limited clinical reports on its effectiveness are available (Reit & Hirsch 1986). Gutta-percha with sealer may be cold condensed (Weine & Gerstein 1976) or chloroform softened (Reit & Hirsch 1986). The introduction of thermoplasticized gutta-percha has also led to greater investigation into the clinical use of gutta-percha for root-end filling (Dawood & Pitt Ford 1989, Sultan & Pitt Ford 1995). Biocompatibility of gutta-percha has been discussed previously.

Zinc oxide–eugenol

Zinc oxide–eugenol cements have been recommended for root-end fillings by clinicians for many decades (Garcia 1937, Nicholls 1965). Dorn & Gartner (1990) showed that root-end fillings with two versions of zinc oxide–eugenol had a significantly higher success rate than with amalgam. In a recent series of histological investigations (Pitt Ford *et al.* 1994, 1995a,b), Super-EBA (Harry J Bosworth Co., Skokie, IL, USA) and IRM (L.D. Caulk Co., Milford, DE, USA) were found to be more biocompatible than other formulations of zinc oxide–eugenol. These fortified versions also have low solubility (Owadally & Pitt Ford 1994), good antibacterial action (Chong *et al.* 1994, Torabinejad *et al.* 1995c) with minimal dye leakage (O'Connor *et al.* 1995). A frequent finding on histological examination has been the presence of giant cells on the surface of the root-end-filling material (Pitt Ford *et al.* 1994, 1995a,b). However, Pantschev *et al.* (1994) did not support the superiority of root-end filling with fortified zinc oxide–eugenol cement (Stailine EBA) over amalgam.

Polymers

Diaket (ESPE-Premier, Norristown, PA, USA), mixed to a thicker consistency than for use as a root-canal sealer, has been advocated as a root-end-filling material (Tetsch 1986). When used as a root-end filling in the study by Williams & Gutmann 1996), the overall healing of the periradicular tissues was found to be favourable. Furthermore, Walia *et al.* (1995) demonstrated in a leakage study that Diaket provided a better seal than either IRM or EBA in both 1 and 3-mm-deep root-end preparations.

Glass ionomer cements

When used as a root-end-filling material the antibacterial effect of glass ionomer cements has been reported as variable (DeSchepper *et al.* 1989, Chong *et al.* 1994) and their sealing ability has been questioned (Pitt Ford & Roberts 1990, Chong *et al.* 1991). However, a favourable periapical tissue response has been reported in the absence of infection in the root canal (Callis & Santini 1987, Pitt Ford & Roberts 1990, Zetterqvist *et al.* 1991). Glass ionomers were found to inhibit the growth of gingival fibroblasts and periodontal ligament cells (Peltola *et al.* 1992, Makkawy *et al.* 1998). This is especially relevant when glass ionomers are used as root perforation materials and are in contact with the periodontium for prolonged periods of time. It has, however, been demonstrated that tissue irritation is not a problem after a period of time (Kolokuris *et al.* 1996).

Amalgam

Amalgam has been the most widely used root-end-filling material for many years (Von Hippel 1914, Block & Bushell 1982, Gutmann & Harrison 1985, Friedman 1991) mainly because dentists are familiar with its handling and because it is radiopaque. In recent years, its continued use has been questioned for reasons such as leakage, biocompatibility, corrosion, staining and overall poor performance (Dorn & Gartner 1990, Pitt Ford *et al.* 1995a). It has come under increasing criticism because of the mercury hazard (Eley & Cox 1993). The tissue response to amalgam root-end fillings has been shown to be unfavourable and associated with inflammation in short-term studies with time periods ranging from 2 weeks to 5 months following placement (Pitt Ford *et al.* 1994, Torabinejad *et al.* 1995b, 1997). Histological staining for mercury has shown traces of amalgam in the tissue some distance from the root-end. These amalgam particles were also associated with inflammation (Pitt Ford *et al.* 1994). In histological studies of root-end fillings amalgam has been associated with the most severe and extensive inflammation of all materials tested including IRM, Super-EBA, Kalzinol (De Trey, Dentsply, Konstanz, Germany), Vitrebond (3M, St. Paul, MN, USA), and Proroot^{TM} MTA (Dentsply, Konstanz, Germany) (Pitt Ford *et al.* 1994, 1995b, Torabinejad *et al.* 1995a, 1997, Chong *et al.* 1997). Polymorphonuclear leucocytes (PMN) were the predominant cells at the 2–5-week time period and lymphocytes the predominant cells at the 10–18-week time period (Torabinejad *et al.* 1995a). No fibrous capsule tissue was seen at either of these time periods. Torabinejad *et al.* (1997) sacrificed experimental monkeys 5 months after surgery and found that lymphocytes were the predominant inflammatory cell in the bulk of the lesions. PMN leucocytes were frequently observed close to the amalgam and a fibrous tissue capsule was present over most amalgam root-end fillings. Clinical and radiographic healing of periapical lesions following apicectomy and amalgam root-end fillings was found to increase from 57% at 1-year recall to 72% at later recalls (2–15 years) (Rud *et al.* 1972). However, Jesslén *et al.* (1995) found that the clinical and radiographic success rate after the placement of amalgam retrofillings decreased from 90% at 1 years to 85% at 5 years, although this effect may not have been due solely to the amalgam. Overall, studies suggest that amalgam shows poor tissue biocompatibility reactions in the short term with improvement in biocompatibility over a longer observation period.

Composite resin

Conventional composite resins contain a polymerizable organic matrix, inorganic reinforcing fillers and a silane-coupling agent which bridges the organic and inorganic components (Ferracane 1995). The organic matrix consists of several (co)monomers (e.g. Bis-GMA, UDMA, EGDMA, TEGDMA, etc.) and various additives which function as (co)initiators, stabilizers or inhibitors. Various factors determine the biocompatibility of a resin-based material, particularly the amount and nature of leachable components (Geurtsen 2000). All organic ingredients of a composite resin are extractable by organic solvents after polymerization. A few components are, however, also leached into an aqueous medium. In particular, considerable amounts of TEGDMA may be released into water. Several composite resins were also found to liberate formaldehyde into water in amounts sufficient to cause local allergic reactions (Øysæd *et al.* 1988, Koch & Staehle 1997).

Geurtsen *et al.* (1998) investigated the cytotoxic effects of 35 single monomers and additives of composite resins in permanent 3T3 cells and primary human oral fibroblasts. Within the groups of (co)monomers and (co)initiators, high or moderate cytotoxic reactions were observed. Proliferation of *Streptococcus sobrinus* and *Lactobacillus acidophilus* was inhibited, promoted or not influenced in a dose-dependent manner by single-resin components in solid- as well as liquid-phase systems (Updegraff *et al.* 1971, Hansel *et al.* 1998). Generally, only very small amounts of these hydrophobic substances are released into an aqueous environment and should not have microbial effects. The severely cytotoxic (co)monomers EGDMA and TEGDMA significantly promoted growth of cariogenic pathogens (Spahl *et al.* 1998). The cytotoxic properties of these (co)monomers together with their microbial growth promotion may contribute to pulpal injury. It has been reported that methyl methacrylate (MMA) may be teratogenic and can cause adverse cardiovascular effects in animals (Phillips *et al.* 1971, Singh *et al.* 1972, Karlsson *et al.* 1995).

Composite resin in combination with a dentine-bonding agent used for root-end filling in a saucer-type preparation has achieved good short- and long-term healing results in clinical studies (Rud *et al.* 1991, 1996a,b). Clinical investigations including patient recalls of up to 9 and 12 years after treatment with Retroplast$^{\mathrm{TM}}$ (Retroplast Trading, Rønne, Denmark) and Gluma$^{®}$ (Bayer AG, Leverkusen, Germany), respectively, showed complete radiographic bone healing over time in a high percentage of cases (Rud *et al.* 1996b, 2001). *In vivo* studies in both monkeys and humans comparing the Retroplast and Gluma combination revealed the absence of inflammatory cells around the root-end filling, with fibroblast and collagen fibres present immediately adjacent to the filling (Rud *et al.* 1996a). In many cases, cementum deposition with Sharpey's fibres were found in intimate contact with the restoration, suggesting a cementogenesis potential for these materials. Unfortunately, absence of complete healing has been found in conjunction with poor haemostatic control during treatment (Rud *et al.* 1991).

More recent composite systems support many of the ideal characteristics for root-end-filling materials and are consequently one of the more common options available for root-end filling. Many resin materials are, however, not suitable for periradicular use and care must be exercised in choosing the right material.

Mineral trioxide aggregate

Mineral trioxide aggregate (MTA) has been proposed as a compound to seal off the pathway of communication between the root-canal system and the periapical tissue and has been shown to have less leakage than amalgam or zinc oxide–eugenol materials in leakage tests (Torabinejad *et al.* 1994, 1995a,b). Other properties that have been investigated include antibacterial effects (Torabinejad *et al.* 1995c), biocompatibility in cell culture (Torabinejad *et al.* 1995d) and when embedded in bone (Torabinejad *et al.* 1995e), its cytotoxicity with the agar overlay and radium chromium release method (Torabinejad *et al.* 1995d) and also in histological examinations of the periapical area associated with root-end fillings in dogs and monkeys (Torabinejad *et al.* 1995a, 1997) and with lateral root perforations in dogs (Holland *et al.* 2001). These tests demonstrated that MTA has good biocompatibity.

The use of MTA as a root-end-filling material in dogs and monkeys provided superior results with considerably less periradicular inflammation compared with amalgam and the production of new cementum over the root-end filling of all the long-term specimens (Torabinejad *et al.* 1995a, 1997). The consistency of response has been unparalleled with other materials. Clinical reports of five cases indicate that MTA may have clinical advantages over other sealers, such as $Ca(OH)_2$-based materials, in the treatment of severe endodontic problems such as root fracture and perforation (Schwartz *et al.* 1999). Histological analysis of the healing of intentional root perforations repaired with MTA (Holland *et al.* 2001) showed no inflammation, with deposition of cementum over MTA in the majority of specimens. All the specimens that were restored with Sealapex exhibited chronic inflammation and only three cases showed slight deposition of cementum over the restorative material.

MTA has also been used as a pulp-capping material for mechanically exposed pulps (Pitt Ford *et al.* 1996), for root-end induction (Tittle *et al.* 1996, Witherspoon & Ham 2001), repair of root perforation (Lee *et al.* 1993, Pitt Ford *et al.* 1995c), and as a barrier during internal bleaching of endodontically treated teeth (Cummings & Torabinejad 1995).

Conclusion

Results from *in vitro* and *in vivo* tests show that endodontic materials possess both beneficial and undesirable properties. The latter may pose a threat to health and/or endodontic treatment outcome by causing local or systemic adverse effects either through direct contact with or leaching of liberated substances into the periodontal tissues and alveolar bone. Biocompatibility is thus as important as physical and chemical features when selecting a material for endodontic therapy. Only those materials that have been proved to have an acceptable biocompatibility in a battery of *in vitro* and *in vivo* tests should be considered for use.

References

Alhadainy HA, Himel VT (1993) Evaluation of the sealing ability of amalgam, Cavit and glass ionomer cement in the repair of furcation perforation. *Oral Surgery, Oral Medicine and Oral Pathology* **75**, 362–6.

Al-Khatib ZZ, Baum RH, Morse DR, Yesilsoy C, Bhambhani S, Furst ML (1990) The antimicrobial effect of various endodontic sealers. *Oral Surgery, Oral Medicine and Oral Pathology* **70**, 784–90.

Araki K, Isaka H, Ishii T, Suda H (1993b) Excretion of ^{14}C-formaldehyde distributed systemically through root canal following pulpectomy. *Endodontics and Dental Traumatology* **9**, 196–9.

Araki K, Suda H, Barbosa SV, Spångberg LS (1993a) Reduced cytotoxicity of a root canal sealer through eugenol substitution. *Journal of Endodontics* **19**, 554–7.

Araki K, Suda H, Spångberg LS (1994) Indirect longitudinal cytotoxicity of root canal sealers on L929 cells and human periodontal ligament fibroblasts. *Journal of Endodontics* **20**, 67–70.

Azar NG, Heidari M, Bahrami ZS, Shokri F (2000) *In vitro* cytotoxicity of a new epoxy resin root canal sealer. *Journal of Endodontics* **26**, 462–5.

Barbosa SV, Burkard DH, Spångberg LSW (1994) Cytotoxic effects of gutta-perch solvents. *Journal of Endodontics* **20**, 6–8.

Becker RM, Hume WR, Wolinsky LE (1983) Release of eugenol from mixtures of ZnOE *in vitro*. *Journal of Pedodontics* **8**, 71–7.

Beck-Mannagetta J, Necek D (1986) Radiologic findings in aspergillosis of the maxillary sinus. *Oral Surgery Oral Medicine Oral Pathology* **62**, 345–9.

Beck-Mannagetta J, Necek D, Grasserbauer M (1986) Dental aspects of solitary maxillary sinus aspergillosis. A clinical, microanalytical and experimental study. *Zeitschrift für Stomatologie.* **83**, 283–315.

Beltes P, Koulaouzidou E, Kotouala V, Kortsaris AH (1995) *In vitro* evaluation of the cytotoxicity of calcium hydroxide-based root canal sealers. *Endodontics and Dental Traumatology* **11**, 245–9.

Bergdahl M, Wennberg A, Spångberg L (1974) Biologic effect of polyisobutylene on bony tissue in guinea pigs. *Scandinavian Journal of Dental Research* **82**, 618–21.

Bezerra Silva LA, Leonardo MR, Faccioloi LH, Figueiredo F (1997) Inflammatory responses to calcium hydroxide-based root canal sealers. *Journal of Endodontics* **23**, 86–90.

Block RM, Bushell A (1982) Retrograde amalgam procedures for mandibular posterior teeth. *Journal of Endodontics* **8**, 107–12.

Block RM, Lewis RD, Hirsch J, Coffey J, Langeland K (1980) Systemic distribution of N2 paste containing ^{14}C *para*formaldehyde following root canal therapy in dogs. *Oral Surgery, Oral Medicine and Oral Pathology* **50**, 350–60.

Boiesen J, Brodin P (1991) Neurotoxic effect of two root canal sealers with calcium hydroxide on rat phrenic nerve *in vitro*. *Endodontics and Dental Traumatology* **7**, 242–5.

Brodin P, Ørstavik D (1983) Effects of therapeutic and pulp protecting materials in nerve transmisiion *in vitro*. *Scandinavian Journal of Dental Research* **91**, 46–50.

Brown BD, Kafrawy AH, Patterson SS (1979) Studies of Sargenti technique of endodontics – autoradiographic and scanning electron microscope studies. *Journal of Endodontics* **5**, 13–9.

Callahan JR (1914) Rosin solution for the sealing of the dentinal tubuli and as an adjuvant in the filling of root canals. *Journal of Allied Dental Society* **9**, 53–63.

Callis PD, Santini A (1987) Tissue response to retrograde fillings in the ferret canine: a comparison of glass ionomer cement and gutta percha with sealer. *Oral Surgery, Oral Medicine and Oral Pathology* **64**, 475–9.

Chong BS, Owadally ID, Pitt Ford TR, Wilson RF (1994) Antibacterial activity of potential retrograde root filling materials. *Endodontics and Dental Traumatology* **10**, 66–70.

Chong BS, Pitt Ford TR, Kariyawasam SP (1997) Tissue resonse to potential root-end-filling materials in infected root canals. *International Endodontic Journal* **30**, 102–14.

Chong BS, Pitt Ford TR, Watson TF (1991) The adaptation and sealing ability of light-cured glass ionomer retrograde fillings. *International Endodontic Journal* **24**, 223–32.

Cohen BI, Pagnillo MK, Musikant BL, Dentsch AS (1998) Evaluation of the release of formaldehyde for three endodontic filling materials. *Oral Health* **88**, 37–9.

Cohen BI, Pagnillo MK, Musikant BL, Deutsch AS (2000) An *in vitro* study of the cytotoxicity of two root canal sealers. *Journal of Endodontics* **26**, 228–9.

Cummings GR, Torabinejad M (1995) Mineral trioxide aggregate (MTA) as an isolating barrier for internal bleaching [Abstract #53]. *Journal of Endodontics* **21**, 228.

Dawood AJ, Pitt Ford TR (1989) Surgical approach to the obturation of apically flared root canals with thermoplasticized gutta-percha. *International Endodontic Journal* **22**, 138–41.

DeSchepper EJ, White RR, Von der Lehr W (1989) Antibacterial effects of glass ionomers. *American Journal of Dentistry* **2**, 50–6.

Dewhirst FE (1980) Structure-activity relationships for inhibition of prostaglandin cyclooxy-oxygenase by phenolic compounds. *Prostaglandins* **20**, 209–22.

Donelly JC (1993) Using a 1-ml syringe for the chloroform dip root canal filling technique. *Journal of Endodontics* **19**, 478.

Dorland's Illustrated Medical Dictionary (1981), 26th edn. Piladelphia, USA: W.B. Saunders Company, p. 355.

Dorn SO, Gartner AH (1990) Retrograde filling materials: a retrospective success–failure study of amalgam, EBA, and IRM. *Journal of Endodontics* **16**, 391–3.

Economides N, Kotsaki-Kovatsi VP, Poulopoulus A, Kolokuris I, Rozos G, Shore R (1995) Experimental study of the biocompatibility of four root canal sealers and their influence on the zinc and calcium content of several tissues. *Journal of Endodontics* **21**, 122–7.

Eley BM, Cox SW (1993) The release, absorption and possible health effects of mercury from dental amalgam: a review of recent findings. *British Dental Journal* **175**, 355–62.

England MC, West NM, Safavi K, Green DB (1980) Tissue lead levels in dogs with RC-2B root canal fillings. *Journal of Endodontics* **6**, 728–30.

Engström B, Spångberg L (1969) Effect of root canal filling material N2 when used for filling after partial pulpectomy. *Svensk Tandläkare Tidskrift* **62**, 815–29.

Ersev H, Schmalz G, Bayirli G, Schweikl H (1999) Cytotoxic and mutagenic potencies of various root-canal-filling materials in eukaryotic and prokaryotic cells *in vitro*. *Journal of Endodontics* **25**, 359–63.

Fehr B, Huwyler T, Wüthrich B (1992) Formaldehyd- and *paraf*-ormaldehyd allergie. *Schweizerische Monatschrift für Zahnmedizin* **102**, 94–6.

Feiglin B (1987) Effect of some endodontic sealers on cell migration in experimental granulomas. *Oral Surgery, Oral Medicine and Oral Pathology* **63**, 371–4.

Ferracane JL (1995) Current trends in dental composites. *Critical Reviews in Oral Biology and Medicine* **6**, 302–18.

Forman GH, Ord RA (1986) Allergic endodontic angiooedema in response to periapical Endomethasone. *British Dental Journal* **160**, 348–50.

Friedman S (1991) Retrograde approaches in endodontic therapy. *Endodontics and Dental Traumatology* **7**, 97–107.

Garcia GF (1937) Apicectomia experimental. *Revista Odontologica* **2**, 145–60.

Geurtsen W (2000) Biocompatibility of resin-modified filling materials. *Critical Reviews of Oral Biology and Medicine* **11**, 333–55.

Geurtsen W, Leinenbach F, Krage T, Leyhausen G (1998) Cytotoxicity of four root canal sealers in permanent 3T3 cells and primary human periodontal ligament fibroblast cultures. *Orals Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **85**, 592–7.

Goldmacher VS, Thilly WG (1983) Formaldehyde is mutagenic for cultured human cells. *Mutation Research* **116**, 417–22.

Grossman LI (1978) Solubility of root canal cements. *Journal of Dental Research* **57**, 927.

Guigand M, Pellen-Mussi P, Le Goff A, Vulcain J-M, Bonnaure-Mallet M (1999) Evaluation of the cytocompatibility of three endodontic materials. *Journal of Endodontics* **25**, 419–23.

Gulati N, Chandra S, Aggarwal PK, Jaiswal JN, Singh M (1991) Cytotoxicity of eugenol in sealer containing zinc oxide. *Endodontics and Dental Traumatology* **7**, 181–5.

Gutmann JL, Harrison JW (1985) Posterior endodontic surgery: anatomical considerations and clinical techniques. *International Endodontic Journal* **18**, 8–34.

Gutmann JL, Rakusin H, Powe R, Bowles WH (1987) Evaluation of heat transfer during root canal obturation with thermoplasticized gutta-percha. Part II. *In vivo* response to heat levels generated. *Journal of Endodontics* **13**, 441–8.

Hansel C, Leyhausen G, Mai UEH, Geurtsen W (1998) Effects of various resin composite (Co) monomers and extracts on two caries-associated micro-organisms *in vitro*. *Journal of Dental Research* **77**, 60–7.

Hansen MG (1998) Relative efficiency of solvents used in endodontics. *Journal of Endodontics* **24**, 38–40.

Heil J, Reifferscheid G, Waldmann P, Leyhausen G, Geurtsen W (1996) Genotoxicity of dental materials. *Mutation Research* **368**, 181–94.

Heling I, Chandler NP (1996) The antimicrobial effect within dentinal tubules of four root canal sealers. *Journal of Endodontics* **22**, 257–9.

Holland R, Filho JA, de Souza V, Nery MJ, Bernabe PF, Dezan E Jr (2001) Mineral trioxide aggregate repair of lateral root perforations. *Journal of Endodontics* **27**, 281–4.

Holland R, Desouza V, Nery MJ, De Mello W, Bernabé PFE, Otoboni Filho JA (1982) Reaction of rat connective tissue to gutta-percha and silver points. A long-term histological study. *Australian Dental Journal* **27**, 224–6.

Holland R, Murata SS, Dezan E, Garlipp O (1996) Apical leakage after root canal filling with an experimental calcium hydroxide gutta-percha point. *Journal of Endodontics* **22**, 71–3.

Hong YC, Wang JT, Hong CY, Brown WE, Chow LC (1991) The periapical tissue reactions to a calcium phosphate cement in teeth of monkeys. *Journal of Biomedical and Material Research* **25**, 485–98.

Huang FM, Tai K-W, Chou M-Y, Chang Y-C (2002) Cytotoxicity of resin-, zinc oxide–eugenol-, and calcium hydroxide-based root canal sealers on human periodontal ligament cells and permanent V79 cells. *International Endodontic Journal* **35**, 153–8.

Hume WR (1984) An analysis of the release and the diffusion through dentin of eugenol from zinc oxide–eugenol mixtures. *Journal of Dental Research* **63**, 881–4.

Hume WR (1988) *In vitro* studies on the local pharmacodynamics, pharmacology and toxicology of eugenol and zinc oxide–eugenol. *International Endodontic Journal* **21**, 130–4.

Jesslén P, Zetterqvist L, Heimdahl A (1995) Long-term results of amalgam versus glass ionomer cement as apical sealant after apicectomy. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **79**, 101–3.

Jukic S, Miletic I, Anic I, Britvic S, Osmak M, Sistig S (2000) The mutagenic potential of AH+ and AH26 by *Salmonella*/microsome assay. *Journal of Endodontics* **26**, 321–4.

Karlsson J, Wendling W, Chen D, Zelinsky J, Jeevanandam V, Hellman S, *et al.* (1995) Methylmethacrylate monomer produces direct relaxation of vascular smooth muscle *in vitro*. *Acta Anaesthesiologica Scandinavica* **39**, 685–9.

Klaiber B, Mittermayer C, Kaden P, Schwechten I (1981) Determination of the toxicity of root-canal-filling materials and their separate components in cell cultures. *Deutsche Zahnärztliche Zeitschrift* **36**, 212–6.

Kobayashi A (1995) Asymptomatic aspergillosis of the maxillary sinus associated with foreign body of endodontic origin. *Journal of Oral and Maxillofacial Surgery* **24**, 243–4.

Koch MJ (1999) Formaldehyde release from root-canal sealers: influence of method. *International Endodontic Journal* **32**, 10–6.

Koch MJ, Staehle HJ (1997) Formaldehyde release from dental materials. *Deutsche Zahnärztliche Zeitskrift* **52**, 778–82.

Kolokuris I, Beltes P, Economides N, Vlemmas I (1996) Experimental study of the biocompatibility of a new glass ionomer root canal sealer (Ketac-Endo). *Journal of Endodontics* **22**, 395–8.

Kolokuris I, Economides N, Beltes P, Vlemmas I (1998a) *In vivo* comparison of the biocompatibility of two root canal sealers implanted into the subcutaneous connective tissue of rats. *Journal of Endodontics* **24**, 82–5.

Kolokuris I, Katsaki-Kovatsi VP, Economides N, Poulouloulos A, Razos G, Vlemmas I (1998b) Influence of zinc oxide and eugenol sealer on concentration of zinc, calcium and copper in rat tissues. *Endodontics and Dental Traumatology* **14**, 210–3.

Koulaouzidou EA, Papazisis KY, Geromichalas GD (1998) Cytotoxicity of three resin-based root canal sealers implanted into the subcutaneous connective tissue of rats. *Endodontics and Dental Traumatology* **14**, 182–5.

Lee SJ, Monsef M, Torabinejad M (1993) Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. *Journal of Endodontics* **19**, 541–4.

Leonardo MR, da Silva LAB, Filho MT, da Silva RS (1999a) Release of formaldehyde by 4 endodontic sealers. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **88**, 221–5.

Leonardo MR, da Silva LA, Almeida WA, Utrilla LS (1999b) Tissue response to an epoxy resin-based root canal sealer. *Endodontics and Dental Traumatology* **15**, 28–32.

Leonardo MR, da Silva LA, Tanomaru Filho M, Bonifacio KC, Ito IY (2000) *In vitro* evaluation of antimicrobial activity of sealers and pastes used in endodontics. *Journal of Endodontics* **26**, 391–4.

Leonardo MR, Silva LA, Utrilla LS, Assed S, Ether SS (1997) Calcium hydroxide root canal sealers – histopathologic evaluation of apical and periapical repair after endodontic treatment. *Journal of Endodontics* **23**, 428–32.

Lewis BB, Chestner SB (1981) Formaldehyde in dentistry: a review of mutagenic and carcinogenic potential. *Journal of the American Dental Association* **103**, 429–34.

Leyhausen G, Heil J, Reifferscheid G, Waldmann Geurtsen W (1999) Genotoxicity and cytotoxicity of the epoxy resin-based root canal sealer AH Plus. *Journal of Endodontics* **25**, 109–13.

Makkawy HA, Koka S, Lavin MT, Ewoldsen NO (1998) Cytotoxicity of root perforation repair materials. *Journal of Endodontics* **24**, 477–9.

Martin H, Martin T (1999) Iodoform gutta percha: MGP, a new endodontic paradigm. *Dentistry Today* **18** (4).

Meryon SD, Brook AM (1990) *In vitro* comparison of the cytotoxicity of twelve endodontic materials using a new technique. *International Endodontic Journal* **23**, 203–10.

Mickel AK, Wright ER (1999) Growth inhibition of *Streptococcus anginosus* (milleri) by three calcium hydroxide sealers and one zinc oxide–eugenol sealer. *Journal of Endodontics* **25**, 34–7.

Munaco FS, Miller WA, Everett MM (1978) A study of long-term toxicity of endodontic materials with use of an *in vitro* model. *Journal of Endodontics* **4**, 151–7.

Nencka D, Walia HD, Austin BP (1995) Histologic evaluation of the biocompatibilty of Diaket [Abstract #716]. *Journal of Dental Research* **74**, 101.

Nicholls E (1965) The role of surgery in endodontics. *British Dental Journal* 59–67.

O'Connor RP, Hutter JW, Roahen JO (1995) Leakage of amalgam and Super-EBA root-end fillings using two preparation techniques and surgical microscopy. *Journal of Endodontics* **21**, 74–8.

Odell E, Pertl CH (1995) Zinc as a growth factor for *Aspergillus* sp. & the antifungal effects of root canal sealers. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **79**, 82–7.

Olsson B, Siwkowski A, Langeland K (1981a) Subcutaneous implantation in the biological evaluation of endodontic material. *Journal of Endodontics* **7**, 355–8.

Olsson B, Siwkowski A, Langeland K (1981b) Intraosseus implantation for biological evaluation of endodontic materials. *Journal of Endodontics* **7**, 253–65.

Olsson B, Wennberg A (1985) Early tissue reaction to endodontic filling materials. *Endodontics and Dental Traumatology* **1**, 138–41.

Ørstavik D, Mjör IA (1988) Histopathology and X-ray microanalysis of the subcutaneous tissue response to endodontic sealers. *Journal of Endodontics* **14**, 13–23.

Osorio RM, Hefti A, Vertucci FJ, Shawley AL (1998) Cytotoxicity of endodontic materials. *Journal of Endodontics* **24**, 91–6.

Owadally ID, Pitt Ford TR (1994) Effect of addition of hydroxyapatite on the physical properties of IRM. *International Endodontic Journal* **27**, 227–32.

Øysæd H, Ruyter IE, Kleven S (1988) Release of formaldehyde from dental composites. *Journal of Dental Research* **67**, 1289–94.

Pantschev A, Carlsson AP, Andersson L (1994) Retrograde root filling with EBA cement or amalgam. A comparative clinical study. *Oral Surgery, Oral Medicine and Oral Pathology* **78**, 101–4.

Pascon EA, Leonardo MR, Safavi K, Langeland K (1991) Tissue reaction to endodontic materials: methods, criteria, assessment, and observations. *Oral Surgery, Oral Medicine and Oral Pathology* **72**, 222–37.

Pascon EA, Spångberg LS (1990) *In vitro* cytotoxicity of root-canal-filling materials. Part 1. Gutta-percha. *Journal of Endodontics* **16**, 429–33.

Peltola M, Salo T, Oikarinen K (1992) Toxic effects of various retrograde root filling materials on gingival fibroblasts and rat sarcoma cells. *Endodontics and Dental Traumatology* **8**, 120–4.

Pertot WJ, Camps J, Remusat M, Proust JP (1992) *In vivo* comparison of the biocompatibility of two root canal sealers implanted into the mandibular bone of rabbits. *Oral Surgery, Oral Medicine and Oral Pathology* **73**, 613–20.

Phillips H, Cole PV, Lettin AW (1971) Cardiovascular effects of implanted acrylic bone cement. *British Medical Journal* **3** (772), 460–1.

Pissiotis E, Sapounas G, Spångberg LS (1991) Silver glass ionomer cement as a retrograde filling material: a study *in vitro*. *Journal of Endodontics* **17**, 225–9.

Pitt Ford TR, Andreasen JO, Dorn SO, Kariyawasam SP (1994) Effect of IRM root end fillings on healing after replantation. *Journal of Endodontics* **20**, 381–5.

Pitt Ford TR, Andreasen JO, Dorn SO, Kariyawasam SP (1995a) Effect of various zinc oxide materials as root-end fillings on healing after replantation. *International Endodontic Journal* **28**, 273–8.

Pitt Ford TR, Andreasen JO, Dorn SO, Kariyawasam SP (1995b) Effect of super-EBA as a root end filling on healing after replantation. *Journal of Endodontics* **21**, 13–5.

Pitt Ford TR, Roberts GJ (1990) Tissue response to glass ionomer retrograde root fillings. *International Endodontic Journal* **23**, 233–8.

Pitt Ford TR, Torabinejad M, Abedi HR, Bakland LK, Kariyawasam SP (1996) Mineral trioxide aggregate as a pulp capping material. *Journal of the American Dental Association* **127**, 491–4.

Pitt Ford TR, Torabinejad M, Hong CU, Kariyawasam SP (1995c) Use of mineral trioxide aggregate for repair of furcal perforations. *Oral Surgery, Oral Medicine and Oral Pathology* **79**, 756–63.

Podbielski A, Boeckh C, Haller B (2000) Growth inhibitory activity of gutta-percha points containing root canal medications on common endodontic bacterial pathogens as determined by an optimized quantitative *in vitro* assay. *Journal of Endodontics* **26**, 398–403.

Reit C, Hirsch J (1986) Surgical endodontic retreatment. *International Endodontic Journal* **19**, 107–12.

Rud J, Andreasen JO, Möller Jensen JE (1972) A follow-up study of 1000 cases treated by endodontic surgery. *International Journal of Oral Surgery* **1**, 215–28.

Rud J, Munksgaard EC, Andreasen JO, Rud V, Asmussen E (1991) Retrograde root filling with composite and a dentin-bonding agent. Part 1. *Endodontics and Dental Traumatology* **7**, 118–25.

Rud J, Rud V, Munksgaard EC (1996a) Retrograde root filling with composite and a dentin-bonding agent. Part 2. *Endodontics and Dental Traumatology* **7**, 126–31.

Rud J, Rud V, Munksgaard EC (1996b) Long-term evaluation of retrograde root filling with dentin-bonding agent. *Endodontics and Dental Traumatology* **22**, 90–3.

Rud J, Rud V, Munksgaard EC (2001) Periapical healing of mandibular molars after root-end sealing with dentine-bonded composite. *International Endodontic Journal* **34**, 285–92.

Saunders EM (1990) *In vivo* findings associated with heat generation during thermomechanical compaction of gutta-percha. Part I. Temperature levels at the external surface of the root. *International Endodontic Journal* **23**, 268–74.

Schmitt W (1951) Die chemischen grundlagenn der erhartenden wurzelfüllungen. *Zahnärztliche Welt* **5**, 560.

Schwartz RS, Mauger M, Clement DJ, Walker WA (1999) Mineral trioxide aggregate: a new material for endodontics. *Journal of the American Dental Association* **130**, 967–75.

Schweikl H, Schmalz G (1991) Evaluation of the mutagenic potential of root canal sealers using the *Salmonella*/microsome assay. *Journal of Material Science: Materials in Medicine* **2**, 181–5.

Schweikl H, Schmalz G, Federlin M (1998) Mutagenicity of the root canal sealer AH plus in the Ames test. *Clinical Oral Investigations* **2**, 125–9.

Schweikl H, Schmalz G, Stimmelmayr H, Bey B (1995) Mutagenicity of AH26 in an *in vitro* mammalian cell mutation assay. *Journal of Endodontics* **21**, 407–10.

Segura JJ, Jmenez-Rubio A (1998) Effect of eugenol on macrophage adhesion *in vitro* to plastic surfaces. *Endodontics and Dental Traumatology* **14**, 72–4.

Serene TP, Vesely J, Boackle RJ (1988) Complement activation as a possible *in vitro* indication of the inflammatory potential of endodontic materials. *Oral Surgery, Oral Medicine and Oral Pathology* **65**, 354–7.

Silver GK, Love RM, Purton DG (1999) Comparison of two root canal obturation techniques: vertical condensation and System B. *International Endodontic Journal* **32**, 287–95.

Silver GK, Taylor TL, Simon JHS (2000) Antimicrobial effect of iodoform gutta-percha cones on *Streptococcus sanguis* and *Enterococcus faecalis*. *New Zealand Endodontic Journal* **26**, 1–3.

Singh AR, Lawrence WH, Autian J (1972) Embryonic–fetal toxicity and teratogenic effects of a group of methacrylate esters in rats. *Journal of Dental Research* **51**, 1632–8.

Sjögren U, Sundqvist G, Nair PNR (1995) Tissue reaction to gutta-percha particles of various sizes when implanted subcutaneously in guinea pigs. *European Journal of Science* **103**, 313–21.

Soares I, Goldberg F, Massone EJ, Soares IM (1990) Periapical tissue response to two calcium hydroxide-containing endodontic sealers. *Journal of Endodontics* **16**, 166–9.

Söderberg TA (1990) Effects of zinc oxide, rosin and resin acids and their combinations on bacterial growth and inflammatory cells. *Scandinavian Journal of Plastic Reconstructive Surgery and Hand Surgery* **24** (S22), 1–87.

Sonat B, Dalat D, Günhan O (1990) Periapical tissue reaction to root fillings with Sealapex. *International Endodontic Journal* **23**, 46–52.

Spahl W, Budzikiewicz H, Geurtsen W (1998) Determination of leachable components from four commercial dental composites by gas and liquid chromatography/mass spectrometry. *Journal of Dentistry* **26**, 137–45.

Spångberg L (1969a) Biological effects of root-canal-filling materials. Part 7. Reaction of bony tissue to implanted root-canal-filling material in guinea pigs. *Odontologisk Tidskrift* **77**, 133–59.

Spångberg L (1969b) Biological effects of root-canal-filling materials. Part 2. Effect *in vitro* of water-soluble components of root-canal-filling materials on HeLa cells. *Odontologisk Revy* **20**, 133–45.

Spångberg L (1969c) Biological effects of root-canal-filling materials. Part 5. Toxic effect of root-canal-filling materials on HeLa cells and human skin fibroblasts. *Odontologisk Revy* **20**, 427–36.

Spångberg L (1969d) Biological effects of root-canal-filling materials. Part 4. Effect *in vitro* of water-soluble components of root-canal-filling materials on HeLa cells. *Odontologisk Revy* **20**, 289–99.

Spångberg L (1974) Biologic effects of root-canal-filling materials. The effect on bone tissue of two formaldehyde-containing root canal filling pastes: N2 and Riebler's paste. *Oral Surgery, Oral Medicine and Oral Pathology* **38**, 934–44.

Spångberg L (1999) Endodontic treatment of teeth without apical periodontitis. In: Ørstavik, D, Pitt Ford, TR, eds. *Essential Endodontology*, 2nd edn. Cambridge, UK: Blackwell Science, p. 228.

Spångberg LSW, Barbosa SV, Lavigne GD (1993) AH26 releases formaldehyde. *Journal of Endodontics* **19**, 596–8.

Spångberg L, Langeland K (1973) Biologic effects of dental materials. 1. Toxicity of root-canal-filling materials on HeLa cells *in vitro*. *Oral Surgery, Oral Medicine and Oral Patholoy* **35**, 402–14.

Staehle HJ, Spiess V, Heinecke A, Muller HP (1995) Effect of root-canal-filling materials containing calcium hydroxide on the alkalinity of root dentin. *Endodontics and Dental Traumatology* **11**, 163–8.

Stea S, Savarino L, Ciabetti G, Cenni E, Stea St Trotta F, Morozzi G, Pizzoferrato A (1994) Mutagenic potential of root canal sealers: evaluation through Ames testing. *Journal of Biomedical and Material Research* **28**, 319–28.

Sultan M, Pitt Ford TR (1995) Ultrasonic preparation and obturation of root-end cavities. *International Endodontic Journal* **28**, 231–8.

Sunzel B, Lazek J, Soderberg TA, Elmros T, Hallmans G, Holm SE (1990) The effect of zinc oxide on *Staphylococcus aureus* and polymorphonuclear cells in a tissue cage model. *Scandinavian Journal of Plastic and Reconstructive Hand Surgery* **24**, 31–5.

Sunzel B, Soderberg TA, Johansson A, Hallmans G, Gref R (1997) The protective effect of zinc on rosin and resin acid toxicity in human polymorphonuclear leukocytes and human gingival fibroblasts *in vitro*. *Journal of Biomedical Material Research* **37**, 20–8.

Sweatman TL, Baumgartner JC, Sakaguchi RL (2001) Radicular temperature associated with thermoplasticized gutta-percha. *Journal of Endodontics* **27**, 512–5.

Swenberg JA, Kerns WD, Mitchell RE, Gralla EJ, Parkow KL (1980) Induction of squamous cell carcinomas of the rat nasal cavity by inhalation exposure to formaldehyde vapors. *Cancer Research* **40**, 3398–402.

Tagger M, Tagger E, Kfir A (1988) Release of calcium and hydroxyl ions from set endodontic sealers containing calcium hydroxide. *Journal of Endodontics* **14**, 588–91.

Telli C, Serper A, Dogan AL, Guc D (1999) Evaluation of the cytotoxicity of calcium phosphate root canal sealers by MTT assay. *Journal of Endodontics* **25**, 811–3.

Tetsch P (1986) *Wurzelspitzenresektionen*, Munich, Hanser. pp. 96–99.

Tittle KW, Farley J, Linkhardt T, Torabinejad M (1996) Apical closure induction using bone growth factors and mineral trioxide aggregate [Abstract #41]. *Journal of Endodontics* **22**, 198.

Torabinejad M, Falah Rastegar A, Kettering JD, Pitt Ford TR (1995b) Bacterial leakage of mineral trioxide aggregate as a root-end-filling material. *Journal of Endodontics* **21**, 109–12.

Torabinejad M, Higa RK, McKendry DJ, Pitt Ford TR (1994) Dye leakage of four root-end-filling materials: effects of blood contamination. *Journal of Endodontics* **20**, 159–63.

Torabinejad M, Hong C-U, Lee S-J, Monsef M, Pitt Ford TR (1995a) Investigation of mineral trioxide aggregate for root-end filling in dogs. *Journal of Endodontics* **21**, 603–8.

Torabinejad M, Hong CU, Pitt Ford TR, Kariyawasam SP (1995e) Tissue reaction to implanted Super-EBA and mineral trioxide aggregate in the mandible of guinea pigs: a preliminary report. *Journal of Endodontics* **21**, 569–71.

Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD (1995c) Antibacterial effects of some root-end-filling materials. *Journal of Endodontics* **21**, 403–6.

Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD (1995d) Cytotoxicity of four root-end-filling materials. *Journal of Endodontics* **21**, 489–92.

Torabinejad M, Pitt Ford TR, McKendry DJ, Abedi HR, Miller DA, Kariyawasam SP (1997) Histologic assessment of mineral trioxide aggregate as a root-end filling in monkeys. *Journal of Endodontics* **23**, 225–8.

Tronstad L, Barnett F, Flax M (1988) Solubility and biocompatibility of calcium hydroxide-containing root canal sealers. *Endodontics and Dental Traumatology* **4**, 152–9.

Uemura M, Hata G, Toda T, Weine FS (1997) Effectiveness of eucalyptol and D-limonene as gutta-percha solvents. *Journal of Endodontics* **23**, 739–41.

Updegraff DM, Chang RWH, Joos RW (1971) Antibacterial activity of dental restorative materials. *Journal of Dental Research* **50**, 382–7.

Vajrabhaya L, Sithisan P (1997) Multilayer and monolayer cell cultures in a cytotoxicity assay of root canal sealers. *International Endodontic Journal* **30**, 141–4.

Vajrabhaya L, Sithisan P, Wilairat P, Leelaphiwat S (1997) Comparison between Sulphorhodamine-B dye staining and 51Cr-release method in cytotoxicity assay of endodontic sealers. *Journal of Endodontics* **23**, 355–7.

Von Hippel R (1914) Zur Technik der Granulomoperation. *Deutsche Monatsschrifte Fürzahnheilkunde* **32**, 255–65.

Walia HD, Newlin S, Austin BP (1995) Electrochemical analysis of retrofilling microleakage in extracted human teeth [Abstract #719]. *Journal of Dental Research* **74**, 101.

Weine FS, Gerstein H (1976) Periapical surgery. In: *Endodontic Therapy*, 2nd edn. St. Louis, USA: Mosby, 287–351.

Wennberg A, Bergdahl M, Spångberg L (1974) Biologic effect of polyisobutylene on HeLa cells and on subcutaneous tissue in guinea pigs. *Scandinavian Journal of Dental Research* **82**, 613–7.

Williams SS, Gutmann JL (1996) Periradicular healing in response to Diaket root-end-filling material with and without tricalcium phosphate. *International Endodontic Journal* **29**, 84–92.

Wilson AD, Batchelor RF (1970) Zinc oxide–eugenol cements. Part II. Study of erosion and disintegration. *Journal of Dental Research* **49**, 593–8.

Witherspoon DE, Ham K (2001) One-visit apexification: technique for inducing root-end barrier formation in apical closures. *Practical Proceedings of Aesthetic Dentistry* **13**, 455–60.

Wolfson EM, Seltzer S (1975) Reaction of rat connective tissue to some gutta-percha formulations. *Journal of Endodontics* **1**, 395–402.

Yesilsoy C, Koren LZ, Morse DR, Kobayashi C (1988) A comparative tissue toxicity evaluation of established and newer root canal sealers. *Oral Surgery, Oral Medicine and Oral Pathology* **65**, 459–67.

Zetterqvist L, Anneroth G, Danin J, Roding K (1988) Microleakage of retrograde fillings – a comparative investigation between amalgam and glass ionomer cement *in vitro*. *International Endodontic Journal* **21**, 1–8.

Zetterqvist L, Anneroth G, Nordenram A (1987) Glass-ionomer cement as retrograde filling material: an experimental investigation in monkeys. *International Journal of Oral and Maxillofacial Surgery* **16**, 459–64.

Zetterqvist L, Hall G, Holmlund A (1991) Apicectomy: a clinical comparison of amalgam and glass ionomer cement as apical sealants. *Oral Surgery, Oral Medicine and Oral Pathology* **71**, 489–91.